Effects of Restraint Stress on Inoculated Tumor Growth and Immune Response in Rats

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ABSTRACT

Rats were given injections s.c. of mammary adenocarcinoma cells which developed into undifferentiated carcinomas within a few days. The animals were either left alone or were stressed by restraint for 3 h a day for 11 days and then left for 12 days undisturbed to recover. During this schedule, some animals were sacrificed immediately after the 11-day stress period, whereas others were allowed the 12-day recovery period; unstressed animals were sacrificed as controls on these 2 days. Tumor burden was significantly increased during stress and markedly decreased after the recovery period as compared to unstressed rats. Higher mitotic activity was seen in the tumors of rats which recovered from stress. The immune system responded differently to stress in healthy and tumor-bearing animals. In the tumor-bearing animals, leukocytes were decreased by stress and increased after the recovery period. Lymphocytes were increased, and neutrophiles and large granular lymphocytes were decreased after the recovery period. Total T-cells and suppressor T-cells were decreased during stress and increased during recovery. The percentage of T-cell populations was unaffected by stress, but the percentage of suppressor T-cells increased during recovery. Natural killer cell activity was unaffected by stress but increased after the recovery period. These results indicate that (a) stress and recovery from stress differentially affect tumor development and growth, (b) stress and recovery from stress cause different effects on the immune system in healthy or tumor-bearing animals, (c) stress and recovery from stress stimuli or inhibit different parts of the immune system, and (d) a decreased lymphocyte count and total and suppressor T-cell numbers correlated best with enhanced tumor growth, whereas increased numbers of neutrophils, large granular lymphocytes, total and suppressor T-cells, natural killer cell activity, and a decreased percentage of T-suppressor cells correlated best with depressed tumor growth.

INTRODUCTION

Stress has been shown to markedly influence incidence, growth, and metastasis, and rejection of chemically induced or implanted tumors (1–6). However, results are contradictory in that both exacerbation or attenuation of tumor development has been reported. For instance, electric shock stress can significantly increase tumor development (1), whereas restraint stress can markedly attenuate tumor growth (5).

These effects are most likely due to changes in the immune system of these animals which have also been shown to be significantly affected by stress. Again, no changes, increases, or decreases of the immune response during stress have been reported. For instance, stress has been reported to increase (6, 7) as well as decrease (8, 9) NK cell activity. Recently, we could show that chronic restraint stress caused leukocytopenia and lymphocytopenia with no effects on T-lymphocytes and NK cell activity but that recovery from stress caused a normalization of leukocytes with significant increases in lymphocytes, T-cells, and NK cell activity (10). Such changes in the immune system can conceivably accelerate or retard tumor development (5).

Since it is difficult to compare immunological and tumor data obtained in animals studied under different experimental conditions, we investigated tumor development and immunological responses to restraint stress in the same animals. Animals received adenocarcinoma cells and were stressed by a chronic restraint for 11 days. Some of these animals were sacrificed immediately after the last stress session or were allowed a recovery period of 12 days and then sacrificed. Tumors were examined, and leukocytes, lymphocytes, T-cell subpopulation, and NK cell activity were measured at these 2 times.

MATERIALS AND METHODS

Animals. Adult female Lewis rats, obtained from Harlan Sprague Dawley, Inc., were used throughout. Housing conditions were already described in detail elsewhere (10).

Stress Protocol. Rats were stressed by forced restraint. This was accomplished by placing the animals in oval metal cages (18 x 6 cm) used to restrain animals for tail vein injections. The legs of the animals were then pulled through the metal rods and secured with plastic tape to the outside of the device. Rats were stressed 3 h daily between 9 a.m. and 12 a.m. for 11 days. During the recovery period, animals were left undisturbed in their home cages for 12 days.

Tumor Induction. Rat mammary adenocarcinoma cells originally induced by i.p. injection of dimethylsininopropionamide (11, 12) were obtained from Dr. E. E. Miller, University of Pennsylvania, School of Medicine, Philadelphia, PA. The tumor cells were cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum, in a humidified atmosphere of 95% air and 5% CO2. Before injection, the tumor cells were washed in growth medium and adjusted to 3 x 106 viable cells/ml.

First, 20 rats were given s.c. injections of increasing doses of tumor cells in order to establish the minimal dose leading to detectable tumors in all animals in 12 days. This dose was found to be 3 x 106 tumor cells per rat. This number of tumor cells in 0.1 ml of medium was then injected s.c. into the back of all rats of this study at Day 0. The animals were then divided into four groups. Groups S1 and S2 were both stressed for 11 days, and Group S1 was sacrificed at this time, whereas Group S2 received a 12-day period of rest in the home cage and was sacrificed thereafter. Groups C1 and C2 were unstressed controls which were

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5 The abbreviations used are: NK, natural killers; LGL, large granular lymphocytes; DMBA, dimethylbenz(a)anthracene.

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sacrificed after 11 (C1) or 23 (C2) days. Animals were weighed and palpated daily for appearance of the tumors. The rats were sacrificed by an i.p. injection of an overdose of ketamine hydrochloride (Bristol Laboratories, Syracuse, NY). Blood was withdrawn from the right heart ventricle into heparinized syringes and used for analysis. The tumors with capsules were gently removed and weighed. Tumor burden was calculated from the tumor weight and rat weight by the following formula.

\[ \% \text{ of tumor burden} = \frac{\text{Tumor wt gain (g)}}{\text{Rat wt (g)}} \times 100 \]

The adrenals, spleen, and livers were removed and weighed.

Pathology. Samples of adrenal gland, liver, spleen, and tumor were fixed in 10% buffered formaldehyde. The tissues were then embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. The sections of adrenal gland, liver, and spleen were studied with light microscope for histological abnormalities. Sections of the tumors were studied for cytological features of the neoplastic cells as well as the degree of necrosis, hemorrhage, fibrosis, lymphocytic infiltration, and mitotic activity. The cases were given code numbers, and the pathologist was not aware of the status of the animals at the time of microscopic examination.

Number of Leukocytes. Number of all leukocytes in 1 mm³ of blood and percentages of neutrophils, lymphocytes, monocytes, and LGL were counted as previously described (10).

Determination of Peripheral Blood T-Lymphocytes. Peripheral blood lymphocytes were obtained by separation on a Ficoll/Hypaque gradient (2), and the lymphocytes were labeled by W3/13, W3/25, and MRC OX8 monoclonal antibodies directed against total T-lymphocytes, T-helper lymphocytes, and T-suppressor lymphocytes, respectively (13). The antibodies were obtained from Sera-Lab. To remove any cross-reactivity of a second antibody, commercially obtained fluorescein isothiocyanate-labeled polyvalent rabbit anti-mouse IgG was adsorbed to rat spleen antibodies. The lymphocytes were obtained by separation on a Ficoll/Hypaque gradient (2), and the lymphocytes were labeled by W3/13, W3/25, and MRC OX8 monoclonal antibodies directed against total T-lymphocytes, T-helper lymphocytes, and T-suppressor lymphocytes, respectively (13). The antibodies were obtained from Sera-Lab. To remove any cross-reactivity of a second antibody, commercially obtained fluorescein isothiocyanate-labeled polyvalent rabbit anti-mouse IgG was adsorbed to rat spleen cells. The percentage of labeled cells was measured on a fluorescence-activated cell sorter (14).

Natural Killer Cell Activity of Spleen Cells. NK activity of spleen cells was measured by the method of $^{51}$Cr release (15). Tumor target cells (YAC-1) and effector cells were incubated and processed as previously described (10). Results were expressed as the percentage of lysis of the target cells according to the following formula.

\[ \% \text{ of lysis} = \frac{\text{Experimental } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}}{\text{Maximum } ^{51}\text{Cr release} - \text{spontaneous } ^{51}\text{Cr release}} \times 100 \]

Statistical Evaluation. All data are expressed as mean ± SE and compared by the rank sum test or Student's t test.

RESULTS

Animals were weighed before and every second day during the experiment until sacrifice. No significant weight differences were found between C1 and S1 as well as C2 and S2.

A palpable tumor was noticed after 7.5 ± 1.3 days in both unstressed groups and after 10.5 ± 1.1 days in both groups of stressed animals. This delay in tumor appearance is statistically significant (P < 0.05).

Tumor weights and tumor burden are shown in Table 1. After 11 days of stress, tumors were slightly but nonsignificantly larger in the unstressed group but significantly decreased in size after the recovery period in the stressed animals. Tumor burden was significantly higher during stress and significantly lower after the recovery period.

Histologically, the tumors were undifferentiated carcinoma (Fig. 1) and did not demonstrate a significant difference in the degree of anaplasia in various groups. Table 2 summarizes the amount of necrosis, fibrosis, mitotic rate, and lymphocytic infiltration in the tumors of various groups. The degree of necrosis and fibrosis correlated with tumor weight: the larger the tumor, the greater the degree of necrosis and fibrosis. Mitotic activity was highest in the S2 group, and this difference was statistically significant when compared to the C2 group. The tumors in Groups C2 and S2 showed a smaller number of lymphocytes in histological sections. These tumors, however, were much larger than those found in Groups C1 and S1. This is in agreement with the generally higher blood lymphocyte count in C2 and S2 groups. No differences in the histology of the adrenal gland, liver, and spleen were apparent among groups.

Table 3 shows weight changes in the adrenal glands, spleens, and livers in unstressed and stressed rats. Restraint stress produced significant increases in adrenal weights which were not significant (P < 0.05).

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Wt of tumors (g)</th>
<th>Tumor burden (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>6.0 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>S1</td>
<td>1.1 ± 0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7 ± 0.2&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>C2</td>
<td>37.4 ± 4.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.4 ± 1.8</td>
</tr>
<tr>
<td>S2</td>
<td>19.2 ± 3.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>7.7 ± 2.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of rats in group.

<sup>b</sup> Mean ± SE.

<sup>c</sup> Comparison of S1 to C1.

<sup>d</sup> Comparison of S2 and C2.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Tumor wt (g)</th>
<th>Lymphocytes&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Necrosis&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Fibrosis&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Mitosis&lt;sup&gt;d&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.8 ± 0.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>145.1 ± 53.3&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.4 ± 0.5&lt;sup&gt;g&lt;/sup&gt;</td>
<td>1.5 ± 0.8&lt;sup&gt;g&lt;/sup&gt;</td>
<td>79.2 ± 25.8&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>S1</td>
<td>1.1 ± 0.5&lt;sup&gt;h&lt;/sup&gt;</td>
<td>206.1 ± 105.7&lt;sup&gt;i&lt;/sup&gt;</td>
<td>1.7 ± 0.8&lt;sup&gt;h&lt;/sup&gt;</td>
<td>2.2 ± 0.8&lt;sup&gt;h&lt;/sup&gt;</td>
<td>58.7 ± 14.5&lt;sup&gt;h&lt;/sup&gt;</td>
</tr>
<tr>
<td>C2</td>
<td>37.4 ± 4.6&lt;sup&gt;j&lt;/sup&gt;</td>
<td>61.9 ± 16.9&lt;sup&gt;k&lt;/sup&gt;</td>
<td>2.9 ± 1.2&lt;sup&gt;l&lt;/sup&gt;</td>
<td>2.5 ± 0.5&lt;sup&gt;l&lt;/sup&gt;</td>
<td>74.5 ± 19.4&lt;sup&gt;l&lt;/sup&gt;</td>
</tr>
<tr>
<td>S2</td>
<td>19.1 ± 3.0&lt;sup&gt;m&lt;/sup&gt;</td>
<td>92.0 ± 76.8&lt;sup&gt;n&lt;/sup&gt;</td>
<td>1.7 ± 1.0&lt;sup&gt;m&lt;/sup&gt;</td>
<td>2.4 ± 0.5&lt;sup&gt;m&lt;/sup&gt;</td>
<td>97.0 ± 16.5&lt;sup&gt;m&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of lymphocytes per 10 oil fields (x1000) of tumor.

<sup>b</sup> Degree of tumor necrosis: 0–25%, 1++; 26–50%, 2++; 51–75%, 3++; 76–100%, 4++.

<sup>c</sup> Degree of fibrosis at the periphery of tumor: mild, 1++; moderate, 2++; marked, 3++.

<sup>d</sup> Number of mitotic figures per 5 high-power fields (x400) of tumor.

<sup>e</sup> Mean ± SE.

<sup>f</sup> Numbers in parentheses, number of rats.

<sup>g</sup> Comparison of S2 and C2 (rank sum test; P < 0.05).
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**Table 3**

Weight (g) of the adrenal gland, spleen, and liver in rats inoculated with mammary carcinoma cells; unstressed (C1; C2) and stressed (S1; S2)

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats/group</th>
<th>Adrenal</th>
<th>Spleen</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>11</td>
<td>0.029 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.53 ± 0.02</td>
<td>7.90 ± 0.56</td>
</tr>
<tr>
<td>S1</td>
<td>11</td>
<td>0.046 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.43 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.01 ± 0.24</td>
</tr>
<tr>
<td>C2</td>
<td>9</td>
<td>0.033 ± 0.001</td>
<td>1.85 ± 0.18</td>
<td>9.92 ± 0.35</td>
</tr>
<tr>
<td>S2</td>
<td>8</td>
<td>0.047 ± 0.003&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.80 ± 0.37</td>
<td>10.21 ± 0.51</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SE.

<sup>b</sup> Comparison of S1 to C1.

<sup>c</sup> Comparison of S2 to C2 (t test; P < 0.05).

**Table 4**

Peripheral blood leukocytes in unstressed (C1; C2) and stressed (S1; S2) female Lewis rats inoculated with tumor cells

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats/group</th>
<th>Leukocytes/mm&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>11</td>
<td>1.0 x 10&lt;sup&gt;4&lt;/sup&gt; ± 9.2 x 10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>S1</td>
<td>11</td>
<td>5.0 x 10&lt;sup&gt;3&lt;/sup&gt; ± 4.9 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>C2</td>
<td>9</td>
<td>2.0 x 10&lt;sup&gt;4&lt;/sup&gt; ± 3.2 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>S2</td>
<td>8</td>
<td>3.2 x 10&lt;sup&gt;4&lt;/sup&gt; ± 5.5 x 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SE.

<sup>b</sup> Comparison of S1 to C1.

<sup>c</sup> Comparison of S2 to C2 (t test; P < 0.05).

The effects of stress on the number of T-lymphocytes are shown in Table 5. Stress decreased significantly the number of total T-cells as determined by W3/13 monoclonal antibody assay and the number of suppressor T-cells as determined by MRC OX8 monoclonal antibody assay. Helper T-cells as determined by W3/25 monoclonal antibody assay were unaffected. After recovery, total T-cells and suppressor T-cells were significantly increased in the previously stressed animals.

**Chart 1.** Effects of stress and recovery on lymphocytes (lymph), neutrophiles (neutro), monocytes (mono), and large granular lymphocytes in tumor-bearing rats. Columns, mean of 6-9 rats; bars, SE. ***, comparison of S1 to C1; ***, comparison of S2 to C2 (t test; P < 0.05). Still elevated after the recovery period. Weights of the spleens were decreased during stress but were similar to those of unstressed animals after the recovery period. Stress had no significant influence on liver weights.

**Table 5**

Number of T-cell populations in blood (mm<sup>3</sup>) of unstressed (C1; C2) and stressed (S1; S2) female Lewis rats inoculated with tumor cells

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of rats/group</th>
<th>W3/13 T-cells</th>
<th>W3/25 T-cells</th>
<th>MRC OX8 T-cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>11</td>
<td>3500 ± 340&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3000 ± 290&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>800 ± 110&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>S1</td>
<td>11</td>
<td>1800 ± 200&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3000 ± 370&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>400 ± 90&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>C2</td>
<td>9</td>
<td>6000 ± 300&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5000 ± 300&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2000 ± 180&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>S2</td>
<td>8</td>
<td>8000 ± 250&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6000 ± 410&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4500 ± 340&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean ± SE.

<sup>b</sup> Comparison of S1 to C1.

<sup>c</sup> Comparison of S2 to C2 (t test; P < 0.05).

**Chart 2.** Effects of stress and recovery on T-cells in tumor-bearing rats. Columns, mean of 6-9 rats; bars, SE. ***, comparison of S2 to C2 (t test; P < 0.05).
in the tumor-bearing animals as determined by W3/13 monoclonal antibody assay during stress or after the recovery period. T-helper cells as determined by W3/25 monoclonal antibody assay were slightly, but nonsignificantly, decreased during stress but significantly reduced after the recovery period. T-suppressor cells as determined by MRC OX8 monoclonal antibody assay were unaffected by stress but increased after the recovery period. No significant influence of stress on the TH/TS ratio was seen, but this ratio was significantly decreased after the recovery period.

Chart 3 shows the effects of stress and recovery on NK cell activity. Stress had no effect on the lysis of YAC-1 cells by spleen cells at three different effector/target cell ratios, but significantly higher activity was found after the recovery period. This enhancement was found at all effector/target cell ratios tested.

**DISCUSSION**

It is generally believed that stress has a negative influence on the immune system and cancer formation. However, anecdotal reports throughout history (16) and published scientific reports show that stress can decrease as well as increase the response of the immune system and can reduce as well as enhance cancer development. These different results are most likely due to differences in the particular immune component studied, the specific tumor investigated, the species of animals used, the specificity and intensity of stress produced, and the time of observation. Thus, only more standardized conditions will result in meaningful correlations among stress, the immune system, and tumor development.

Exacerbations of cancer processes by stress have been demonstrated by a number of experiments. Mice receiving p815 mastocytoma and inescapable electric shock showed tumors earlier and died sooner than mice which remained unstressed (17). Tumor rejection of rats receiving Walker 256 sarcoma and inescapable electric shock was lower than that seen in unstressed controls (18). Perhaps more interesting are reports indicating a beneficial effect of stress on tumor development. Significant inhibition in the growth and development of DMBA-induced mammary tumors was found by exposure of the animals to immobilization, electric shock, and an overcrowding-sound situation (19–21). Animals exposed to electroconvulsive shock and cold showed a better rejection and reduced growth of murine sarcoma and lymphoma tumors (1, 3). Our laboratory found also that immobilization stress decreased the growth of DMBA-induced mammary tumors (5, 22). Among many factors, the intensity and duration of stress seem to play a major role. In our experiments on tumor induction by DMBA, we find a protective effect only if the animals are stressed after the third injection of the carcinogen, whereas no protection was observed if stress began before the first injection. Similarly, a 20-day foot shock had no effect on the number of DMBA-induced tumors, whereas a 40- or 80-day footshock period significantly reduced the number of these tumors (23).

Similarly, different results are obtained by studying the immune system under stress. Enhanced as well as depressed responses have been reported. Thus, stress has been reported to reduce the number of leukocytes and lymphocytes, the percentage of T- and B-cells, natural killer cell activity, and the lymphocyte response to phytohemagglutinin stimulation (6, 24). However, stress has also been shown to increase serum γ-globulin, macrophage toxicity, interferon levels, and natural killer cell activity (7, 25). Again, different experimental conditions are most likely the cause of those contradictory results.

Due to described differences in experimental conditions, it is not only difficult to assess the individual effects of stress on the immune system or tumor development, it becomes almost impossible to find correlations among stress, the immune system, and cancer development. For this reason, we studied tumor formation and the immune response in the same animals during and after stress.

In this study we used tumor cells obtained from dimethylas-indinopropionamide-induced tumors in female Sprague Dawley rats. Injection of 3 x 10^6 cells produced a tumor in all rats given injections. The tumor appeared after about 7 days in an animal and reached a weight of about 25 g in 2–3 wk. The tumors were identified as undifferentiated carcinoma. The tumors appeared slower and were smaller but grew faster during the initial stress period. After recovery from stress, tumor development was reduced, and tumors found were significantly smaller than those found in unstressed controls. Under these conditions, initial stress delayed tumor appearance but promoted tumor development, whereas recovery from stress significantly retarded tumor growth. Histological evaluations of tumors obtained from control and stressed rats during and after restraint were similar with the exception of significantly increased mitotic activity in the group which recovered from stress. This could be interpreted that tumor growth is slowed by keeping the cells longer in the mitotic phase, but an acceleration of tumor destruction by the immune system cannot be ruled out. The weights of the adrenal were increased during and after stress. The weight of the spleen was decreased during stress only.

Immunologically, the number of leukocytes increased significantly during and after stress. The percentage of lymphocytes decreased only after recovery, whereas the percentages of neutrophiles and LGL had increased at this time. The number of monocytes decreased during stress and after recovery as compared to unstressed groups. No change in total T-cells was seen, but the percentage of helper cells decreased, and the percentage of suppressor cells increased after recovery only. Similarly, NK cell activity was only significantly increased after recovery from stress.

Since immunological investigations were performed in these same tumor-bearing animals, comparison between tumor growth and changes in individual components of the immune system can be performed. The delayed tumor appearance and possibly higher tumor weights during stress do not seem to correlate to any immunological parameters measured except a correlation between a decrease in monocytes and a slight increase in tumor weight. However, the retarded growth of the tumor after the recovery period correlates well with signs of a stimulated immune system at this time as evidenced by an increase in the number of neutrophils, LGLs, and NK cell activity. The involvement of T-cells is unclear, since at this time the percentage of helper cells decreased but that of the suppressor cells increased. The reduction of T-helper cells may perhaps be due to the mechanical stress factor of the tumors.

Since we have examined the immune system under identical conditions in healthy rats (10), it is worthwhile to compare the immunological data of tumor-bearing rats with those of nontu-
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The presence of the tumor seems to significantly influence the activity of the immune system. In healthy rats, the number of leukocytes is $1 \times 10^6$ cells in 1 mm$^3$. This number drops to $3.2 \times 10^4$ cells in 1 mm$^3$ in tumor-bearing animals. The percentage of lymphocytes is reduced from 66–52%, and the percentage of neutrophils is increased from 31–47% in the presence of a tumor. Monocytes, LGLs, T-cell subpopulations, and NK activity are unaffected by the tumor. Differences between healthy and tumor-bearing rats are more pronounced during and after stress. Healthy rats bearing no tumor have a leukocyte count of about $1 \times 10^4$ cells/mm$^3$ of blood which decreased to $4 \times 10^3$ after 11 days of stress and returned to normal after the recovery period. In tumor-bearing rats, leukocytes behaved similarly during stress but were higher ($3 \times 10^4$/mm$^3$) after the recovery period. The percentage of lymphocytes decreased markedly in healthy animals during stress (66% versus 30%) but not in tumor-bearing animals (72% versus 76%). In contrast, the percentage increased after recovery in the healthy animals (30% versus 52%) but decreased in the tumor-bearing animals (52% versus 42%). In healthy animals, neutrophils increased during stress (32% versus 71%) and decreased after the recovery period (71% versus 18%); in tumor-bearing animals, stress had no effect (22% versus 20%), and the recovery period increased the percentage of neutrophils (20% versus 55%). Monocytes of healthy animals were unaffected by stress or recovery but were decreased in tumor-bearing animals (3% versus 1.5%). LGLs are increased during stress (2% versus 7%) and recovery in healthy rats; in tumor-bearing animals, LGLs increase by stress (3% versus 9%) and remain unchanged after recovery (9% versus 7%). Total T-cells and helper suppressor T-cells were unaffected by stress in healthy and tumor-bearing rats. However, total and helper T-cells increased after the recovery period in healthy (70% versus 90% and 45% versus 56%) as well as tumor-bearing animals (40% versus 62% and 28% versus 38%); helper T-cells are unaffected in healthy and tumor-bearing animals. In healthy and tumor-bearing animals, NK cell activity is unaffected by stress but increased after recovery. These comparisons show that parts of the immune system respond differently to stress in animals which bear or do not bear a tumor and that results obtained with healthy animals cannot be readily extrapolated to tumor-bearing animals.

It is interesting to speculate how stress affects the immune system and tumor growth. The release of corticosterone during stress must certainly inhibit the immune system and promote tumor growth. However, corticosterone secretion will only play a role during the initial stress exposure, where it would depress the immune system, but steroid levels will gradually decrease and decreases could retard tumor growth as seen in this study. The release of enkephalins and endorphins during stress can decrease or enhance the immune system (9, 27). Enhancement of the endorphin/enkephalin system may be responsible for the enhanced number of NK cells and/or rate of cytotoxicity. Unfortunately, no measures of endorphins and enkephalins are available under our experimental conditions. Thus, further measurements of these conditions to obtain correlations between the immune response and hormonal events during and after stress.

In conclusion, stress and recovery from stress cause different effects on the immune system and tumor growth. This probably explains some of the controversial data published in the literature. To more fully understand the effects of stress on the immune system and tumor development, precise time studies should be undertaken, and measurements of different biochemical, immunological, and pathological parameters must be obtained in the same animals.

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REFERENCES


![Fig. 1. Photomicrograph of the neoplasm, showing sheets of anaplastic malignant cells with pleomorphic nuclei and prominent nucleoli. Note atypical mitotic figures and several lymphocytes scattered throughout the tumor. H & E, × 400.](image-url)
Effects of Restraint Stress on Inoculated Tumor Growth and Immune Response in Rats


*Cancer Res* 1985;45:5128-5133.